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(54) Title: CHROMOSOME BANDING BY MONOCLONAL ANTIBODIES TO NUCLEAR PROTEINS

(57) Abstract

Disclosed is a method for determining morphologically distinct regions of chromosomes by providing an antibody to a distinct chromosomal protein, where the antibody will bind to the protein in the chromosome in a pattern, contacting the antibody with the chromosome, and determining the pattern in which the antibody binds to the protein. Banding patterns substantially identical to R-banding and C-banding may be obtained with the use of the present invention. Also disclosed is the use of the labeled antibodies to mark chromosomes for flow cytometry, and the use of the labeled antibodies in conjunction with cytohybridization techniques using DNA or RNA probes, in order to determine the chromosomal location of the genetic material with which the probes hybridize.

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CHROMOSOME BANDING BY MONOCLONAL ANTIBODIES TO NUCLEAR PROTEINS

Background of the Invention

The present invention relates to generation, utilization, and visualization of chromosome banding patterns utilizing antibodies to nuclear proteins.

The development of the science of cytogenetics has been facilitated by the discovery that reproducible staining patterns can be seen when chromosomes are treated with various dyes and other agents. These patterns, appearing as longitudinal bands on the chromosomes, add a new dimension of structural detail. The banding patterns for each chromosome in the cell complement are unique, and these unique patterns permit the unambiguous identification of each chromosome for which a pattern is known. Changes in normal chromosomal structure are characteristic of certain disease states, and banding patterns on chromosomes make these changes easier to identify, thus improving the efficiency of diagnosis. The ability to visualize chromosomal detail is also useful in basic investigation of chromosomal organization and function because changes in banding correlate with processes of DNA replication and transcription (D. Comings, Ann. Rev. Genet. Cytogenet. 6, 59 (1952)).

Chromosomal banding was first reported by Caspersson in 1968 (Exp. Cell Res. 49, 219), using a fluorescing drug (quinacrine) as a staining agent. Later, Pardue (Science, 168,1356 (1970)) described a similar procedure using the common Giemsa dye. The advantage of the Giemsa procedure is that the banding pattern can be visualized under an ordinary light microscope, while banding produced by quinacrine can only be seen under a special fluorescence microscope. The two procedures give similar banding which are now standard reference patterns, Q-banding and G-banding.

In 1971, Dutrillaux and Lejuene noted that treatment of

a chromosomal preparation with hot saline solution prior to Giemsa treatment produced a pattern, R-banding, that is the reverse of G-banding; thus, bands that appear pale in G-banding appear dark in R-banding.

Preparations treated with a denaturing agent, such as acid, alkali, or hot sodium citrate before Giemsa staining, exhibit dark staining located primarily at the centromeres. The pattern is designated C-banding because it is believed to identify constitutive or structural chromosomal material.

Various other drugs and dyes have been used to produce banding patterns. These agents share a common structural feature (a planar ring structure), but the chemical basis for their binding to chromosomes is obscure. For this reason the precise mechanism of chromosomal G, C, and R-banding is also unknown; however, the differential staining pattern is thought to correlate with variations in base composition of the DNA. Quinacrine and Giemsa normally bind to regions where adenine and thymine predominate (A-T rich); other agents, such as the antibiotic chromomycin A, bind preferentially to regions where guanine and cytosine predominate (G-C rich) (G. Prantera, Science 264, 79 (1979)). The distinction is significant because A-T rich regions are functionally different from G-C rich regions.

Antibodies are used routinely in immunohistology to map antigen distributions in cells and tissues because they bind with high specificity and can be conveniently labeled and detected. Dev et al., Exptl. Cell Res. 74, 288 (1972) used antibodies to a DNA base in a similar way to band human chromosomes. After cell preparations were denatured with formamide, they were exposed to anti-adenosine which was then visualized by an indirect fluorescence procedure. The banding pattern is similar to that seen with quinacrine or Giemsa dye.

Conventional banding reagents, despite widespread

acceptance and use, nevertheless are somewhat less than satisfactory. One particular disadvantage of conventional chemistry is that it produces banding patterns that differ depending on the visualizing dye used, on temperature and storage conditions of the reagents, and on other variables. Thus, it is not always possible to compare banding patterns from one lot of dye with those of another. As a result, there is a need for a banding technique that will give consistent, repeatable results and staining densities in order to facilitate direct comparisons of banding patterns obtained from different lots of reagents, under different conditions, or by different researchers.

It is believed that banding patterns associated with and indicative of the distribution of chromosomal protein (rather than the DNA bases) are of interest because these proteins presumably have regulatory purposes, and their distribution as revealed by such staining patterns could shed light on chromosomal organization and function. Monoclonal antibodies are ideal agents for this purpose because their high specificity should produce high resolution patterns.

Accordingly, a principal object of this invention is to provide methods for using monoclonal antibodies having specificity for chromosomal proteins to visualize those proteins.

Another object of the invention is to provide a method for using monoclonal antibodies to determine chromosomal structure and organization.

Brief Description of the Invention

In accordance with the foregoing objectives, the present invention provides a method for defining or visualizing morphologically distinct regions of chromosomes, which comprises obtaining a monoclonal antibody to a specific chromosomal protein having a detectable label attached thereto, treating a preparation

of chromosomes with the label, and determining the pattern in which the labeled monoclonal antibody is bound to the chromosomes.

The invention further provides a method of karyotyping chromosomes by defining the structure of the chromosomes according to the method above and characterizing the organization of the chromosomes in a cell according to number and type expressed in terms of those structures. In a preferred embodiment, the method is applied to mammalian chromosomes, and in a particularly preferred embodiment it is applied to human chromosomes.

In accordance with another object of the invention, the monoclonal antibodies are labeled with either a fluorescent molecule or an enzyme. In a preferred embodiment, the monoclonal antibody is labeled with a peroxidase enzyme.

In accordance with another aspect of the present invention, there is provided a method for determining chromosomal structure using a monoclonal antibody having a specificity for a chromosomal protein of approximately 175,000 daltons which is localized in the pericentromeric region, which antibody binds to chromosomes in a pattern similar to that of C-banding.

There is also provided a method which comprises the use of a labeled monoclonal antibody to a chromosomal protein having a molecular weight of 38,000 daltons, which antibody binds to chromosomes in a pattern similar to that of R-banding.

There is further provided a method for marking morphologically distinct bands on chromosomes which comprises obtaining at least one monoclonal antibody to a specific homogenous monoclonal protein, attaching a label to the antibody, and contacting the labeled monoclonal antibody with a preparation of chromosomes, whereby the chromosomes are labeled at distinct sites.

The invention also provides a method for determining the location of a chromosomal region of interest by combining the steps of generating a banding pattern (as set forth above) with the steps of obtaining a labeled RNA or DNA probe to a region of the chromosomes, hybridizing the probe with that region, and determining the location of the hybridized probe in relationship to the pattern.

Detailed Description of the Invention

Chromosomal protein-specific antibodies suitable for use in the present invention may be developed using conventional techniques to any particular chromosomal protein of interest. The particular chromosomal protein to which the antibody is specific will, of course, determine the type of banding pattern that is produced by the use of the present invention.

The present invention contemplates the use of antibodies selected from a large number of possible antibodies to nuclear proteins. Indeed, it is believed that there are several as yet unrecognized nuclear proteins associated with chromosomes to which antibodies can be developed through techniques such as those set forth herein. Moreover, as will be recognized by persons skilled in the art, a large number of monoclonal antibodies can be developed to any particular protein of interest. Accordingly, although the present invention is described in the context of particular preferred antibodies that have already been developed, other suitable antibodies may be developed and utilized in a similar manner.

In accordance with preferred embodiments of the invention, the method for creating and visualizing chromosomal banding patterns comprises the use of one or the other of two monoclonal preferred antibodies whose specificities for subregions of human chromosomes make them particularly suitable for use as probes in a variety of cytogenetic procedures. One monoclonal antibody (Mab

244-7) has a specificity for the relatively gene-rich regions that stain dark in the R-banding procedure. The other monoclonal antibody (Mab 898-9) has a specificity for a protein that is preferentially located in pericentromeric regions of the human genome that stain darkly by the C-banding technique. Both antibodies are of the IgM heavy chain isotype and have been purified in high concentration from the fluid of ascites tumor in pristane-primed mice. In accordance with other preferred embodiments, any antibody to one or the other of these two proteins may be utilized.

The hybridoma clones that produce these preferred antibodies were developed from the fusion of NS-1 mouse myeloma cells and splenocytes from Balb/c mice hyperimmunized with nuclear preparations of human cells. A total of 24 monoclonal antibody-producing hybridoma lines raised in response to nuclear antigens were screened for staining reactions with paraformaldehyde-fixed human epidermal cells and with human metaphase chromosomes prepared for human cytogenetic analysis and fixed in 3:1 methanol/acetic acid. The immunizing antigen preparation used to produce clone 898-9 was obtained from pokeweed-mitogen-stimulated peripheral blood lymphocytes and that used to produce clone 244-7 was from acute lymphoblastic leukemic cells. The preparation of these antibodies has been described in the literature. See A. Epstein and C. Clevenger, Progress in Nonhistone Protein Research, Vol.1, pp.117-137 (I. Bekhor, ed. 1985), which is incorporated herein by this reference. The hybridomas (clones 244-7 and 898-9) utilized in the production of the two preferred monoclonal antibodies are maintained in permanent collection by the inventors.

Similar immunization, fusion, and screening techniques can be utilized to develop other hybridomas which produce monoclonal antibodies suitable for use in the present invention.

Immunoblot analysis indicates that Mab 244-7 recognizes a nuclear protein of 38,000 daltons (p38), while Mab 898-9 recognizes a nuclear protein of 175,000 daltons. In accordance with preferred embodiment of the present invention, the monoclonal antibody used in the method is an antibody to the 38,000 dalton protein recognized by Mab 244-7 or the 175,000 dalton protein recognized by Mab 898-9.

Localization of both Mab 244-7 and Mab 898-9 in human cells was determined using two immunohistochemical methods: indirect immunofluorescence, and avidin-biotin-peroxidase complex (ABC) staining.

Indirect immunofluorescence studies on paraformaldehyde-acetone fixed cell preparations treated with Mab 244-7 demonstrate an intense nuclear rim staining pattern in interphase cells and bright fluorescence of chromosomes during mitosis. Similar studies on normal and malignant human cells treated with Mab 898-9 show dense diffuse nuclear and nucleolar staining. Both antibodies stain condensing chromosomes during mitosis and appear to cross-react with an antigen associated with intermediate filaments.

These findings were confirmed when human epidermoid carcinoma cells of the A-431 line were treated with antibodies and the banding patterns of the chromosomes visualized by means of the ABC technique. Mab 244-7 is seen localized in the periphery of the interphase cell nucleus as well as in condensing prophase and metaphase chromosomes; Mab 898-9 is localized more diffusely in the interphase nucleus with areas of concentration around nucleoli. This antibody also stains condensing chromosomes.

Both the 244-7 and the 898-9 antibodies were applied to metaphase chromosomes that had been prepared by standard cytogenetic procedures and fixed in 3:1 methanol:acetic acid. Banding patterns were visualized as before using the

ABC technique. In the human karyotype, Mab 244-7 identifies an antigen whose distribution on chromosomes is identical to that of R-bands. Metaphase cells treated with Mab 244-7 display a medium resolution (500-band) karyotype, while prometaphase chromosomes, similarly treated, display very fine (800-band) R-banding. Mab 898-9 preferentially recognizes regions of centromeric heterochromatin in the human karyotype, although its antigen is apparently distributed throughout the chromosome complement.

Studies of the banding patterns of both Mab 244-7 and 898-9 in preparations of hamster and mouse metaphase chromosomes indicate that the antigens recognized are conserved in evolution, although the banding pattern on rodent chromosomes is less distinct than that on humans. Each antibody also stains plant chromosomes.

Surprisingly, the banding patterns achieved with the present invention can exhibit significantly greater detail than those generated by conventional techniques. (This is particularly true of the R-banding patterns generated by Mab 244-7.) At the same time, the correspondence between the banding patterns generated by the use of the present invention and conventional R- and C-banding patterns is striking. Thus, the techniques of the present invention can, to a large extent, be used as replacement techniques for conventional banding techniques, while providing greater resolution, more consistent results (because of the monoclonal nature of the reagents), and superior reproducibility by others.

The role of the specific protein (p38) which Mab 244-7 detects is unknown, but its distribution in the R-band regions suggests an association with the structural or functional characteristics of this region of the karyotype. The R-band regions are gene-rich as compared to the G-banded regions, and that may be the reason they are less well tolerated as chromosomal imbalances. The studies of Goldman et al. (Science 224:686 (1984)) indicate that

G-bands include genes for specialized functions, whose natural state is one of generalized suppression, and R-bands include genes for housekeeping functions which are in an early-replicating, transcription-competent state. A protein having the R-band distribution similar to p38 would be required to maintain the proper chromosome infrastructure. Monoclonal antibody 244-7, which uniquely binds this protein, is a useful tool in exploring its biological role.

Monoclonal antibodies 244-7 and 898-9 can be used as probes or labels for many cytogenetic applications. In addition to the basic application in karyotyping, they may be used as indicators in flow cytometry, image analysis, and in heterochromatic studies.

The antibodies of the present invention may be labeled with any convenient detectable species, including but not limited to radioisotopes, enzymes, fluorescent molecules, or electron-dense moieties. The detecting species may be bound to the antibodies by conventional procedures well known to those in the art.

Alternatively, the antibodies may be detected by a second antibody such as an anti-mouse IgM antibody which is itself labeled with a detectable species.

These antibodies may also be detected using the ABC (avidin-biotin complex) technique, a type of second antibody procedure which greatly amplifies the detectable signal by binding more enzyme molecules to each antibody molecule. The procedure is described in product literature supplied by the manufacturer, Vector Labs of Burlingame, California.

The use of Mab 244-7, in combination with either direct or indirect immunofluorescence or the ABC staining technique, provides karyotypic definition that is superior to other conventional R-banding procedures, because of the high resolution bands it produces. The resolution of this technique is greater than that of radioactive phosphate

banding or any other procedures using antibodies (e.g. Dutrillaux et al., C.R. Acad. Sci. Paris Ser. D272, 2638 (1971)).

Some suitable techniques according to the present invention will be set forth in Examples 1-4, which follow. Those techniques include R-banding, C-banding, analysis of banded chromosomes with flow cytometry, and *in situ* hybridization techniques used to map genetic probes to specific chromosomal sites.

Briefly, the banding techniques involve contacting an appropriate antibody to a nuclear protein with a chromosome preparation and permitting the antibody to become bound to the protein of interest. The antibody may be labeled either before or after it becomes bound to the protein, by any of the numerous conventional antibody labeling techniques. Such labels may be chromophores, enzymes, radiolabels, or any other desired label. After the labeling and binding steps, the chromosomes may be visualized. This visualization step may include introducing a colorimetric or fluorescent substrate for the enzyme label, exposing photographic material to the radiolabel, or direct visualization of a visible label, all according to well known techniques. The banding patterns may be analyzed in order to karyotype the chromosomes, or for other purposes, by visual techniques, photographic techniques, or instrumental image analysis techniques.

The flow cytometry techniques described herein may be practiced with any of the many suitable flow cytometry instruments which are now widely available. The flow cytometry methods of the present invention permit sorting of chromosomal material stained or labeled with the antibodies of the present invention, by virtue of the nonhomogeneous nature of the binding of the antibodies to the chromosomes. This, in turn, permits differentiation of the individual chromosomes with flow cytometry in a manner that is well understood.

The present invention may be applied to the identification, location, and mapping of specific genes on the chromosome by utilizing the anti-nuclear antibodies of the present invention in conjunction with RNA or DNA hybridization techniques. In this method, suitable RNA or DNA probe to the gene or region of interest is provided. That probe is labeled using a technique that will permit the location of the probe following hybridization to be determined. Radiolabeling is a particularly preferred labeling technique. The probe is then permitted to hybridize with the genetic material to be analyzed. The genetic material is also brought in contact with an antibody to a nuclear protein of the type described herein to generate a banding pattern. Thereafter, the hybridization site can be determined in the context of the banding pattern by any suitable technique. Where the probe is radiolabeled, a photographic emulsion sensitive to the radiation emitted by the radiolabel can be brought in contact with the hybridized genetic material, thereby exposing the emulsion over the hybridization site. When the emulsion is developed, the exposed portions become visible and their locations can be correlated with the banding pattern.

Example 1: R-Banding of Human Chromosomes Stained with Monoclonal Antibody 244-7.

Normal human peripheral blood lymphocytes are mitogenically stimulated with phytohemagglutinin (PHA) for four days in RPMI-1640 medium containing 10% fetal calf serum and antibiotics. One hour before harvest, the cells are incubated with 1.0 μg/ml colcemide to arrest the cells in metaphase. The cells are then harvested in a centrifuge tube and spun at 800 rpm for eight minutes. After aspirating the supernatant, the cells are gently resuspended in 5 ml of 0.075 M hypotonic KCl solution drop by drop and incubated for 5 minutes at 4°C. The cells

are again centrifuged at 600 rpm for eight minutes and resuspended in 3 ml of freshly prepared fixative (3 parts methanol and 1 part glacial acetic acid) drop by drop. After five minutes, the cells are spun for 5 minutes at 600 rpm and fixed two additional times as above. After the third fixation step, the cells are resuspended in 10-20 drops of fixative and dropped onto glass slides held at a 45° angle and precooled at 20°C. One or two drops are used per slide. After shaking the slides dry, they are ready for staining.

For immunostaining, the slides are incubated with Mab 244-7 (IgM) supernatant diluted 1:25 with phosphate buffered saline (PBS) for two hours at room temperature. After rinsing with PBS, the slides are stained with the avidin-biotin complex immunoperoxidase technique (Vector Laboratories, Burlingame, CA) using 3,3'-diaminobenzidine as the colorimetric reagent. The slides are mounted with a coverslip and examined under oil immersion light microscopy. The chromosomes seen in metaphase spreads exhibit an R-banding pattern with a resolution of up to 500 bands. If pre-synchronized cells are used, high resolution banding of up to 800 bands can be visualized by this technique. By photographing appropriate metaphase spreads, karyotypes can be prepared to identify the presence or absence of chromosomal abnormalities. In addition, slides prepared in this manner may be used in conjunction with image analysis equipment to produce karyotypes and detailed information on chromosome morphology for research and clinical applications.

Example 2: C-Banding of Human Chromosomes Stained with Monoclonal Antibody 898-9.

Chromosome preparations are prepared as described in Example 1. Slides are incubated with supernatant containing monoclonal antibody 898-9 (IgM) diluted 1:25 with PBS for 2 hours at room temperature. After rinsing

with PBS, the slides are stained with the avidin-biotin complex immunoperoxidase procedure (Vector Laboratories, Burlingame, CA) using 3,3'-diaminobenzidine as the colorimetric reagent. The slides are mounted with a coverslip and examined under oil immersion light microscopy. The chromosomes seen in metaphase spreads exhibit a C-banding pattern. By photographing appropriate metaphase spreads, the chromosomes can be used for cytogenetic analysis. In addition, the slides may be used in conjunction with image analysis equipment to study chromosome morphology for research and clinical application.

Example 3: Flow Cytometric Analysis of R-Banded Human Chromosomes Stained with Monoclonal Antibody 244-7.

Four day PHA stimulated cultures of human peripheral blood lymphocytes are harvested by centrifugation at 1000 rpm for 10 minutes. The cell pellet is resuspended in 0.075 M KCl and placed on ice for five minutes. This suspension of swollen mitotic cells is centrifuged at 750 rpm for 10 minutes and the pellet is resuspended in fixative containing 3 parts methanol and 1 part glacial acetic acid as described in Example 1. After 3 changes of fixative the cells are resuspended in 5 ml of polyamine buffer (15 mM Tris-HCl, 2mM EDTA, 0.5 mM EGTA, 20 mM NaCl, 0.2 mM spermine, 0.5 mM spermidine, 14 mM 2-mercaptoethanol, pH 7.2) at 4°C. The cells are then centrifuged at 1500 rpm for 2 minutes and resuspended in 1-2 ml of polyamine buffer containing 0.1% digitonin. The suspension is held on a vortex mixer for 30 seconds at high speed to lyse the mitotic cells and liberate the chromosomes. The chromosomes are then stained in solution with fluorescein-labeled monoclonal antibody 244-7 and propidium iodide and analyzed on a flow cytometer using dual fluorescence excitation filters (green for Mab 244-7 staining, red for propidium iodide). The brightly green

fluorescent bands produced by Mab 244-7 staining represent R-bands and are used to karyotype or sort human chromosomes for research and clinical applications.

Example 4: R-Banding of Chromosomes by Mab 244-7 for *In Situ* Hybridization Studies

In situ hybridization allows for the direct detection of specific DNA sequences on chromosomes. This method is therefore useful for mapping specific genes to their chromosomal location. To identify which chromosome contains the hybridization site, the follow procedure is performed. (This procedure is modified from Harper, M.E. and Saunders, G.F.: Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization, Chromosoma 83:431-439 (1981)).

Human metaphase chromosomes are prepared on slide preparations as described above in Example 1. Slide preparations are treated with 100 µg/ml RNase A in 2x SSC buffer (1xSSC=0.15 M NaCl, 0.015M Na citrate, pH 7.0) for 60 min. at 37°C, then immersed in 70% formamide 2x SSC buffer pH 7.0 at 70°C for 2 minutes to denature the chromosomal DNA. Radiolabeled DNA probes are then applied, coverslips mounted with rubber cement, and incubation continued at 37°C for 16 hours. Slides are then rinsed in three changes of 50% formamide in 2x SSC buffer at 39°C to remove nonspecifically bound DNA, and then are rinsed thoroughly in 2x SSC buffer at 39°C. Hybridized slides are then coated with Kodak NTB_2 brand photographic emulsion, diluted 1:1 with water, sealed, and stored desiccated at 4°C for 5-22 days. Autoradiographs are developed in Kodak Dektol brand photographic developer, for 2 minutes at 15°C, fixed in Kodak brand fixer for 5 minutes, rinsed well, and air dried.

For immunostaining, slides are reacted with Mab 244-7 supernatant diluted 1:25 in PBS for 2 hours at room temperature. After rinsing with PBS, the slides are

stained with the avidin-biotin complex immunoperoxidase procedure (Vector Laboratories, Burlingame, CA) using 3,3'-diaminobenzidine as the colorimetric reagent.

The slides then mounted with a coverslip and examined under oil immersion light microscopy. Chromosomes will show an R-banding pattern and areas of radiolabeled DNA hybridization will show silver granules over specific chromosomal locations. The chromosomal location of the DNA under study can then be determined by the R-banding pattern of the chromosome it overlies.

Although the present invention has been described in the context of certain preferred embodiments and preferred monoclonal antibodies, it will be apparent to those of ordinary skill in the art that other antibodies may be used and that conventional modifications of the reagents and techniques described herein are possible. Accordingly, it is intended that the scope of the protection afforded hereby be determined in reference to the claims that follow.

WHAT IS CLAIMED IS:

1. A method for determining morphologically distinct regions of chromosomes, comprising:

obtaining a monoclonal antibody to a chromosomal protein, wherein said antibody is capable of binding to said proteins in banding patterns;

contacting said antibody with a preparation of chromosomes to permit said antibodies to bind to said protein in a pattern; and

determining the pattern in which said labeled monoclonal antibody binds to said chromosomes.

2. A method according to Claim 1, further comprising the steps of:

karyotyping the chromosomes by characterizing the organization of said chromosomes in a cell according to number and type expressed in terms of said structure.

3. A method of analyzing chromosomes, comprising:

obtaining a monoclonal antibody which binds to a specific homogeneous chromosomal protein;

contacting said monoclonal antibody with a preparation of chromosomes, whereby said antibody binds to said chromosomes;

providing a label on said antibody so that said chromosomes are labeled at morphologically distinct sites; and

analyzing a suspension of said labeled chromosomes by flow cytometry, whereby homologues of chromosome types are identified, classified and separately resolved according to the intensity and distribution of said label on said homologues.

4. A method according to Claim 1, 2, or 3 wherein said chromosomes are mammalian chromosomes.

5. A method according to Claim 4, wherein said chromosomes are human chromosomes.

6. A method according to Claim 1, 2, or 3 wherein said monoclonal antibody is labeled with a fluorescent molecule.

7. A method according to Claim 1 or 2 wherein said monoclonal antibody is detected by a second antibody and said second antibody is labeled with a fluorescent molecule.

8. A method according to Claim 1, 2, or 3, wherein said monoclonal antibody is labeled with an enzyme.

9. A method according to Claim 7, wherein said monoclonal antibody is labeled with a peroxidase enzyme.

10. A method according to Claim 7, wherein each said molecule of antibody is joined to an enzyme by means of molecules of avidin and biotin.

11. A method according to Claim 10, wherein said monoclonal antibody binds to chromosomes in a pattern substantially identical to that of C-banding.

12. A method according to Claim 5, wherein said monoclonal antibody has a specificity for a chromosomal protein of approximately 175,000 daltons which is associated with the pericentromeric region.

13. A method according to Claim 5, wherein said monoclonal antibody has a specificity for a protein of approximately 38,000 daltons and binds to chromosomes in a pattern substantially identical to that of R-banding.

14. A method according to Claim 1, further comprising the steps of:

obtaining a labeled RNA or DNA probe to a region of said chromosomes;

hybridizing said probe with said region; and

determining the location of said hybridized probe in relationship to said pattern.

15. A method for marking morphologically distinct bands on chromosomes, comprising:

obtaining at least one monoclonal antibody species to a specific homogeneous chromosomal protein, said monoclonal antibody being labeled;

contacting said labeled monoclonal antibody with a preparation of chromosomes, whereby said chromosomes are labeled at distinct sites.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/03397

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12Q 1/68. G01N 33/53

U.S. Cl.: 435/6,7

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/6, 7, 28; 436/501, 510, 513, 518, 548; 935/81, 110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Journal of Cell Biology, Volume 88, published in 1981 (Rockefeller University Press, New York, NY) G. C. Howard et al., "Monoclonal Antibodies Against a Specific Nonhistone Chromosomal Protein of Drosophila associated with active genes," pages 219-225, see especially the abstract on page 219.	1,6,15
X	Chromosoma (Berl.) Volume 80, published in 1980 (Berlin, Germany), H. Saumweber, et al., "Monoclonal Antibodies Against Chromosomal Proteins of Drosophila melanogaster, "pages 253-275, especially lines 1-7 on page 255.	1,6,15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 January 1989	23 MAR 1989
International Searching Authority	Signature of Authorized Officer
ISA/US	Ardin Marschel

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chromosoma (Berl.) Volume 85, published in 1982 (Berlin, Germany), R. Kabisch et al., "Evolutionary Changes in Nonhistone Chromosomal Proteins within the Drosophila melanogaster Group revealed by Monoclonal Antibodies, "pages 531-538, see especially the abstract on page 531.	1,6,7, 15
X	Chromosoma (Bel.), Volume 88, published in 1983 (Berlin, Germany), A. Dangli et al., "Differential distribution of Nonhistone proteins from polytene chromosomes of Drosophila melanogaster after heat shock, "pages 201-207, see especially abstract on pages 201.	1,6,7, 15
Y	M. Jamrich, "Chromosome Structure and Function," published 1986, by Van Nostrand Reinhold Co., (New York, NY), see pages 221 and 226-228.	1-15
Y	US, A, 4,594,318 (GUSELLA, ET AL.) 10 June 1986, See Figures 1 and 2 and Claim 1.	1-15
A	Chromosoma (Berl.), Volume 83, published in 1981 (Berlin, Germany), M. E. Harper, et al., "Localization of Single Copy DNA Sequences on G-banded Human Chromosomes by in situ Hybridization," pages 431-439.	14
A	Science, Volume 168, published in 1970 by the American Association for the Advancement of Science (Washington D.C.), M. L. Pardue et al., "Chromosomal Localization of Mouse Satellite DNA," pages 1356-1358.	14

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	Proceedings of the National Academy of Science (USA), Volume 76, Number 3 published in 1979 by the National Academy of Science (Washington D.C), A. V. Carrano et al., "Measurement and Purification of Human Chromosomes by Flow Cytometry and Sorting," pages 1382-1384.	2-13
A	Experimental Cell Research, Volume 74, published in 1972 by Academic Press, Inc. (Uppsala, Sweden), V. G. Dev et al., "Consistent Pattern of Binding of Anti-Adenosine Antibodies to Human metaphase chromosomes," pages 288-293	1-15
A	A. Epstein et al., "Progress in Non-Histone Protein Research," Volume 1, published in 1985, by CRC Press Inc. (Baton Rouge, Florida), pages 117-137.	1-15
A	Annual Review of Genetics, Volume 12, published in 1978 by Annual Review Inc. (Palo Alto, California), D. Comings, "Mechanisms of Chromosome Banding and Implications for Chromosome Structure," pages 25-46	1-15
A	P. Benn et al., "Human Cytogenetics," published in 1986 by IRL Press (Washington D.C.), page 57-84	1-15